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Efficacy of *Trichoderma* spp. against *Sclerotium rolfsii*

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Fifteen isolates of *Trichoderma* spp. were isolated in Trichoderma Specific Meidum (TSM) from soils of different betelvine plantations of West Bengal. Cultural, morphometric and antagonistic potential against *S. rolfsii* (5 isolates) of each *Trichoderma* isolates were studied. The morphometric studies revealed that the highest number of phialides, phialospores, chlamydospore were recorded in isolate T_{13} and length of conidiophore was reordered in T_9, T_4, T_{12} isolates. The chlamydospores recorded were in twins, chains or in intercalary in positions. From the cultural and morphometric characters, it was revealed that among the 15 isolates of *Trichoderma* spp., T_6, T_7, T_9, T_{10}, T_{11}, T_{12}, and T_{13} are *Trichoderma viride*, T_4 is *T. virens* and rest isolates are *T. harzianum*. The antagonistic potential of *Trichoderma* isolates against *Sclerotium rolfsii* showed that T_9 and T_{10} had highest promise under *in vitro* conditions (Dual Plate Technique) by fully overgrowing the pathogens as reordered by Bell's scale within 7-8 days.

Key words : Biocontrol, *Piper betle, Trichoderma viride, T. harzianum, T. virens, Sclerotium rolfsii.*

INTRODUCTION

The betelvine (*Piper betle*) is cultivated under moist, sub-tropical and differed light conditions with adequate supply of moisture in the soil. These conditions are congenial for many root and aerial pathogens that scourge this vine. Among various severe diseases foot rot of betelvine caused by *Sclerotium rolfsi* has been found most devastating. (Chattopadhyay and Maiti, 1990)

In last three decades, a lot of researches have been carried out on the antagonistic nature of several species of *Trichoderma* (Papavizas, 1985; Chet, 1987), they have shown highest potential against many soil borne fungal pathogens. Researches on *T. harzianum* and *T. viride* as a biocontrol agent also show differential antagonistic potential among isolates (Maity and Sen, 1985; Biswas, 1999; D'Souza *et al.*, 2001). Our emphasis in the present studies is on the need for screening specific isolates of antagonists against various isolates of *S. rolfisii.* Several antagonistic isolates of *Trichoderma* sp. are collected from different betelvine gardens of West Bengal and they are then tested under *in vitro* conditions against the pathogen *S. rolfisii* which causes the foot rot disease of betelvine.

MATERIALS AND METHODS

Isolates of Trichoderma sp. from soil

Fifteen different isolates of *Trichoderma* spp. were randomly isolated from soils which were collected from different barojes of West Bengal by dilution plate technique using TSM (Trichoderma Specific Medium) (Elad and Chet, 1983) modified by Saha and Pan (1997) (Table 1). All the isolates were maintained on PDA slants at 5°C.

Table 1 : Source of isolates of *Trichoderma* spp.

Isolates	Place of Colection
T_1	Plant Virus Research Farm, BCKV, Kalyani, Nadia (Baroj-1)
T_2	Plant Virus Research Farm, BCKV, Kalyani, Nadia (Baroj-2)
T_3	Plant Virus Research Farm, BCKV, Kalyani, Nadia (Baroj-3)
T_4	Mondauri Farm, BCKV, Mondauri,

	North 24 Parganas (Baroj-1)
T_5	Mondauri Farm, BCKV, Mondauri, North 24 Parganas (Baroj-2)
T_6	Rautari, Nadia
T_7	Simurali, Nadia
T_8	Simurali, Nadia
T_9	Rautari, Nadia
T_{10}	Simurali, Nadia
T_{11}	Rautari, Nadia
T_{12}	Simurali, Nadia
T_{13}	Rautari, Nadia
T_{14}	Rautari, Nadia
T_{15}	Simurali, Nadia

Isolates of Sclerotium rolfsii from soil

Five different isolates of *Sclerotium rolfsii* were isolated from infected stem which were collected from different barojes of West Bengal using PDA medium (Potato Dextrose Agar) (Table 2). All the isolates were maintained on PDA slants at 5°C.

Table 2 : Source of isolates of *Sclerotium rolfsii*.

Isolates	Place of Collection
S_1	Plant Virus Research Farm, BCKV, Kalyani, Nadia (Baroj-1)
S_2	Plant Virus Research Farm, BCKV, Kalyani, Nadia (Baroj-2)
S_3	Simurali, Nadia
S_4	Simurali, Nadia
S_5	Rautari, Nadia

General characteristic of Trichoderma isolates

Micrometric measurement of phialospores and phialides was done by mounting 4 day old young culture in lactophenol stained with cotton blue and observed under high power research microscope. Micrometric measurement of chlamydospores was made from one month old culture following the method described earlier. The length-breadth ratios of phialospares, phialides and chlamydospores were recorded.

Antagonistic potential of Trichoderma isolates

The antagonistic potential of 15 isolates of *Trichoderma* were tested on PDA medium by dual culture plate technique. An amount 5 day-old culture of *S. rolfisii* was plated aseptically at the edge of Petri plates 2 days before the placement of an amount of culture of *Trichoderma* sp. Paired cultures in the Petri plate were incubated and observed for 9 days before being discarded. All the ratings were done after contacts between pathogen and antagonist using a modified Bell's (Bell *et al.*, 1982) scale (1-5) developed as follows :

Class I-The antagonist completely overgrew the pathogen (100% overgrowth);

Class II-The antagonist overgrew at least 2/3rd of pathogen surface (75% overgrowth);

Class III-The antagonist colonized on half the growth of the pathogen (50% overgrowth);

Class IV-The pathogen and antagonist locked at the point of contact; and

Class V-The pathogen overgrew the antagonist.

RESULTS AND DISCUSSION

Identity of the isolates of Trichoderma

The identity of test isolates of antagonist was attempted through a study of cultural and morphometic characters. The colony characters of fifteen isolates on PDA as observed visually at different time intervals (24-96 hrs) were recorded (Table 3). In general, colony morphology of all the isolates was more or less similar showing sparse to thin cottony mycelial mass with whitish border. Sporulation started after 48 hrs of incubation at 28±1°C for all the isolates. These observations on colony characters showed no difference from those made earlier by Rifai (1969), Domsch *et al.* (1980), Martha (1992), Majumdar (1993) and D'Souza *et al.* (2001).

The micrometric measurements (Table 4) showed that the largest phialospore was produced by isolate T_4 and it ranged from 3.75-7.50 (5.62) µm and smallest was produced by isolates T_2, T_5, T_{10} T_{11}, T_{13}, T_{14}, (length ranged from 2.5-3.50 µm and breadth ranged from 2.26-2.59 µm). The length : breadth ratio was found to be highest in T_{13}.

The length of phialides ranged between 10-13.75 µm and width ranged between 2.66-3.10 µm. The longest phialides was produced by T_9 [12.5-15 µm (13.75)] and largest by also T_9 (12.5-15 µm x 2.5x-3 µm) and smallest phialids was produced by T_{12} (6.25-10.25*2.71-2.99 µm). The length: breadth ratio was highest in T_9 (5:1) where as smallest in T_{10} (1.83 : 1) (Table 4).

The morphometric characters and micrometric measurements of 15 isolates of *Trichoderma* spp. revealed that T_6, T_7, T_9, T_{10}, T_{11}, T_{12} and T_{13} isolates

Table 3 : Colony characters of fifteen isolates of *Trichoderma* spp.

Isolate	Growth after 24 hrs.	Growth after 48 hrs.	Growth after 72 hrs.	Growth after 96 hrs.
T_1	White sparse growth.	White cottony mycelial growth.	Greenish white mycelial growth.	Light green coloured full plate growth.
T_2	Whlte sparse growth.	White fluffy mycelial growth.	Full plate growth, whitish, non-sporulation.	Light green coloured full plate growth.
T_3	White cottony growth.	2/3plate white cottony growth.	Full mycelial growth, greenish coloured, sporulation at the older region.	Deep green sporulation all over the plate.
T_4	White sparce growth.	Some as 24 hrs, growth, white.	Full plate growth, greenish appearance to the periphery of the disc.	Dark green full plate growth.
T_5	White cottony appearance .	A raised growth pattern having whitish cottony mycelia growth.	Cottony, compact, light greenish growth.	Deep greenish sporulation, full plate mycelia growth.
T_6	Off-white mycelial growth.	White sparce growth, 2/3 of plate.	Full plate growth, whitish green growth at the periphery of the plate.	Dirty green coloured full plate growth.
T_7	White mycelial growth around the disc.	White sparce growth, no sporulation.	Full plate growth, greenish white mycelial growth.	Light green coloured sporulation, full plate growth.
T_8	White thin growth over the medium.	Round, white growth over the medium.	Light greenish sporulation surrounding the inoculated disc.	Light green coloured full plate growth.
T_9	White cottony appearance	Cottony mycelial growth, Light yellowish tinge,	Greenish white low sporulation, light yellowish tinge.	Light greenish compact full plate growth.
T_{10}	Fluffy cottony appearance	Same as 24 hrs growth, mycelia cover 2/3 of plate.	Full plate growth, light greenish appearance in entire plate.	Compact yellowish green growth.
T_{11}	White cottony mycelial growth.	White cottony growth on the surface of medium.	Whitish green sporulation at 2/3 of the plate.	Deep green sporulation all over the medium.
T_{12}	White sparse growth.	Cottony growth over the medium.	Whitish green appearance nearly entire medium.	Dirty green sporulation all over the medium.
T_{13}	White fluffy growth.	White slow growth rate.	Very light greenish growth, mycelia cover 2/3 of the plate.	Deep green sporlation.
T_{14}	White cottony appearance	Sparse growth, cover 2/3 of the medium.	Light greenish appearance from centre to the periphery of the plate.	Deep green sporulation.
T_{15}	Bright white mycelia growth.	Cottony, white mycelia growth. Covers 2/3 of the plate.	Full plate compact, cottony growth, sporulation. More or less in entire plate.	Deep green, compact sporulation

Table 4 : Colony characters of fifteen isolates of *Trichoderma* spp.

Isolate No.	Phialospore/Conidia (µm)*			Phialide (µm)*			Chlamydospore (µm)*		
	L	B	L.B. ratio	L	B	L.B. ratio	L	B	L.B. ratio
T_1	2.50-3.75 (3.12)[1]	2.12-3.32 (2.72)	1.14:1	6.1-12.5 (9.3)	2.47-3.10 (2.78)	3.34:1	6.53-9.05 (7.79)	5.44-8.59 (7.01)	1.11:1
T_2	2.75-3.25 (3.0)	2.37-2.82 (2.59)	1.15:1	7.5-15.0 (11.25)	2.97-3.16 (3.06)	3.67:1	8.96-16.28 (12.62)	6.44-15.47 (10.95)	1.15:1
T_3	3.0-4.0 (3.5)	2.62-3.5 (3.09)	1.13:1	7.5-12.5 (10.00)	2.92-4.0 (3.46)	2.89:1	7.62-12.20 (9.91)	6.31-11.99 (9.15)	1.08:1
T_4	3.75-7.50 (5.62)	3.39-7.06 (5.22)	1.07:1	7.5-15 (11.25)	2.16-3.10 (2.63)	4.27:1	11.21-22.23 (16.72)	7.02-17.48 (12.25)	1.36:1
T_5	2.5-2.75 (2.62)	2.14-2.38 (2.26)	1.15:1	10-15 (12.5)	2.66-2.79 (2.72)	4.53:1	9.25-16.78 (13.01)	8.46-16.32 (12.39)	1.05:1
T_6	2.5-5.0 (3.75)	2.14-4.61 (3.15)	1.06:1	5-12.5 (8.75)	2.46-3.66 (3.06)	2.85:1	6.72-9.88 (8.3)	6.72-9.7 (8.21)	1.01:1
T_7	2.5-4.25 (3.37)	2.12-3.88 (3.0)	1.12:1	6.25-10 (8.12)	2.51-3.39 (2.95)	1.75:1	5.26-10.55 (7.90)	4.48-10.23 (7.35)	1.07:1
T_8	2.75-4.25 (3.50)	2.39-3.88 (3.13)	1.11:1	7.5-10.25 (8.87)	2.20-2.99 (2.59)	3.42:1	12.62-32.16 (22.39)	11.23-28.55 (19.89)	1.12:1
T_9	2.5-4.25 (3.37)	2.07-2.88 (2.97)	1.13:1	12.5-15 (13.75)	2.51-3 (2.75) 2.92-3.9	5.0:1	12.61-17.02 (14.81)	10.49-13.03 (11.76)	1.25:1
T_{10}	2.5-3.25 (2.87)	2.07-2.86 (2.46)	1.16:1	5-7.5 (6.25)	(3.41) 2.16-2.66	1.83:1	6.14-15.15 (10.64)	5.72-11.36 (8.54)	1.24:1
T_{11}	2.5-3.5 (3.0)	2.12-3.06 (2.58)	1.16:1	7.5-12.5 (10.00)	(2.41) 2.71-2.99	4.41:1	15.11-24.97 (20.04)	11.19-16.0 (13.59)	1.47:1
T_{12}	3.5-5.25 (4.37)	3.12-4.81 (3.96)	1.10:1	6.25-10.25 (8.25)	(2.85) 2.11-2.77	2.89:1	5.11-11.18 (8.14)	4.25-9.42 (6.83)	1.19:1
T_{13}	2.5-3.0 (2.75)	2.12-2.57 (2.34)	1.17:1	5-12.5 (8.75)	(2.44) 2.78-2.96	3.58:1	18.51-26.48 (22.49)	11.33-14.98 (13.15)	1.71:1
T_{14}	2.5-3.0 (2.75)	2.14-2.88 (2.51)	1.14:1	7.5-18.75 (13.12)	(2.87) 2.51-3.12	4.57:1	13.46-15.68 (14.57)	10.90-13.20 (12.05)	1.20:1
T_{15}	2.5-4.5 (3.5)	2.12-4.13 (3.12)	1.12:1	5-13.75 (9.37)	(2.81)	3.33:1	12.42-16.80 (14.61)	10.40-16.39 (13.41)	1.08:1

* Average of three replications [1]Figure in parentheses are average of ten observations

are *Trichoderma viride*, T_4 isolate is *T. virens* and rest isolates are *T. harzianum*.

Antagonistic potential of antagonist isolates against test pathogens

The result (Table 5) showed that 2 isolates of *Trichoderma viride.*, T_9 and T_{10} were highly antagonistic to *S. rolfsii*, totally overgrew over the pathogenic organism within 7-8 days. Those isolates were categorized in Class I according to Bell's scale. The other *Trichoderma* sp. isolates gave an altogether different picture. T_{11}, T_{14}, T_7, T_8, rated as R_2, and T_1, T_2, T_3, T_4, T_5, T_6, T_{15} were rated as R_3 whereas T_{12}, T_{13} were rated as R_4.

Trichoderma sp. specifically *Trichoderma viride* (T_9, T_{10}) reached in Class I stage within 7-8 days of isolations. However, based on this information the antagonistic *T. viride*, did not allow an easy selection of isolates as the variability in the antagonistic characteristic within isolates and isolate-pathogen was very high. But the antagonistic isolates. T_9 and T_{10} appeared to be a nearly assureded choice due to their effective action against *S. rolfsii*.

It is well known that there is sufficient selectivity of isolates of *T. viride* in their antagonistic efficiency towards a particular pathogen (Papavizas and Lumsden, 1980; Cook and Baker, 1983). Maiti *et al.* (1982) obtained clear variation in antagonistic poten-

Table 5 : Screening of *Trichodrama* sp. isolates against *S. rolfsii*

Isolate S_1 of *Sclerotium rolfsii*

Isolate	Point of Contact (days)	Bell's scale after (days)					
		4th	5th	6th	7th	8th	9th
T_1	3	R_4	R_4	R_4-R_3	R_3-R_2	R_2	R_2
T_2	3	R_4	R_4-R_3	R_3	R_3	R_3	R_3
T_3	3	R_4	R_4-R_3	R_4-R_3	R_3	R_3	R_3
T_4	3	R_4	R_4-R_3	R_3	R_3	R_3	R_3
T_5	3	R_4	R_3	R_3	R_3	R_3	R_3
T_6	3	R_4	R_4-R_3	R_4-R_3	R_3	R_3	R_3
T_7	3	R_4-R_3	R_3	R_3	R_3	R_3	R_3
T_8	3	R_4	R_4	R_3	R_3	R_3	R_3
T_9	3	R_4-R_3	R_3-R_2	R_3-R_2	R_1	R_1	R_1
T_{10}	3	R_4-R_3	R_3-R_2	R_3-R_2	R_2	R_1	R_1
T_{11}	3	R_4	R_3	R_3	R_3-R_2	R_2	R_2
T_{12}	3	R_4	R_3	R_3	R_3	R_3	R_3
T_{13}	3	R_4	R_4	R_3	R_3	R_3	R_3
T_{14}	3	R_4-R_4	R_3	R_3	R_3	R_2	R_2
T_{15}	3	R_4-R_3	R_4-R_3	R_3	R_2	R_2	R_2

Isolate S_2 of *Sclerotium rolfsii*

Isolate	Point of Contact (days)	Bell's scale after (days)					
		4th	5th	6th	7th	8th	9th
T_1	3	R_3	R_3-R_2	R_3-R_2	R_2	R_2	R_2
T_2	3	R_4	R_4-R_3	R_3	R_3	R_3	R_3
T_3	3	R_4	R_4	R_4-R_3	R_3	R_3	R_3
T_4	3	R_4-R_3	R_4-R_3	R_3	R_3	R_3	R_3
T_5	3	R_4	R_4-R_3	R_3	R_3	R_3	R_3
T_6	3	R_4	R_3	R_3	R_3	R_3	R_3
T_7	3	R_4	R_3	R_3	R_3-R_2	R_2	R_2
T_8	3	R_4-R_3	R_4-R_3	R_3	R_3	R_3	R_3
T_9	3	R_3	R_3	R_3-R_2	R_2	R_2	R_1
T_{10}	3	R_3	R_3-R_2	R_3	R_2	R_2	R_1
T_{11}	3	R_3	R_3	R_3-R_2	R_3-R_2	R_2	R_2
T_{12}	3	R_4	R_4-R_3	R_4-R_3	R_3	R_3	R_3
T_{13}	3	R_4	R_4	R_4-R_3	R_3	R_3	R_3
T_{14}	3	R_3	R_3	R_3-R_2	R_2	R_2	R_2
T_{15}	3	R_4	R_4-R_3	R_3	R_2	R_2	R_2

Isolate S_3 of *Sclerotium rolfsii*

Isolate	Point of Contact (days)	Bell's scale after (days)					
		4th	5th	6th	7th	8th	9th
T_1	3	R_4	R_4	R_3	R_3	R_3	R_3
T_2	3	R_4	R_4-R_3	R_3	R_3	R_3	R_3
T_3	3	R_4	R_4	R_4-R_3	R_3	R_3	R_3
T_4	3	R_4-R_3	R_4-R_3	R_3	R_3	R_3	R_3
T_5	3	R_4	R_4-R_3	R_3	R_3	R_3	R_3
T_6	3	R_4	R_3	R_3	R_3	R_3	R_3
T_7	3	R_4-R_3	R_4-R_3	R_3	R_2	R_2	R_2
T_8	3	R_4-R_3	R_3	R_3	R_3-R_2	R_2	R_2
T_9	3	R_3	R_2	R_2	R_1	R_1	R_1
T_{10}	3	R_3	R_3-R_2	R_2	R_2	R_1	R_1
T_{11}	3	R_4	R_4-R_3	R_3	R_3-R_2	R_2	R_2
T_{12}	3	R_4	R_4-R_3	R_4-R_3	R_3	R_3	R_3
T_{13}	3	R_4	R_4	R_4-R_3	R_3	R_3	R_3
T_{14}	3	R_4-R_3	R_4-R_3	R_3	R_3-R_2	R_2	R_2
T_{15}	3	R_4	R_4	R_4-R_3	R_2	R_3	R_3

Isolate S_4 of *Sclerotium rolfsii*

Isolate	Point of Contact (days)	Bell's scale after (days)					
		4th	5th	6th	7th	8th	9th
T_1	3	R_4	R_4	R_4	R_4-R_3	R_3	R_3
T_2	3	R_4	R_4	R_4	R_4-R_3	R_3	R_3
T_3	3	R_4	R_4-R_3	R_4-R_3	R_3	R_3	R_3
T_4	3	R_4	R_4	R_3	R_3	R_3	R_3
T_5	3	R_4	R_4-R_3	R_3	R_3	R_3	R_3
T_6	3	R_4	R_3	R_3	R_3	R_3	R_3
T_7	3	R_3	R_3-R_2	R_2	R_2	R_2	R_2
T_8	3	R_3	R_2	R_2	R_2	R_2	R_2
T_9	3	R_3	R_3	R_2	R_1	R_1	R_1
T_{10}	3	R_3	R_3	R_2	R_2-R_1	R_1	R_1
T_{11}	3	R_3	R_3	R_3-R_2	R_1	R_1	R_1
T_{12}	3	R_4	R_3	R_3	R_3-R_2	R_2	R_2
T_{13}	3	R_4	R_4-R_3	R_3	R_3	R_3	R_3
T_{14}	3	R_4	R_3	R_3-R_2	R_2	R_2	R_1
T_{15}	3	R_4	R_3	R_3	R_3	R_3	R_3

Isolate S_5 of *Sclerotium rolfsii*

Isolate	Point of Contact (days)	Bell's scale after (days)					
		4th	5th	6th	7th	8th	9th
T_1	3	R_4	R_4-R_3	R_4-R_3	R_3	R_3	R_3
T_2	3	R_4	R_4-R_3	R_3	R_3	R_3	R_3
T_3	3	R_4	R_4-R_3	R_4-R_3	R_3	R_3	R_3
T_4	3	R_4	R_4	R_4	R_4-R_3	R_3	R_3
T_5	3	R_4	R_4	R_4-R_3	R_4-R_3	R_3	R_3
T_6	3	R_4	R_4	R_3	R_3	R_3	R_3
T_7	3	R_4	R_4-R_3	R_3	R_3-R_2	R_2	R_2
T_8	3	R_3	R_3	R_3-R_2	R_2	R_2	R_2
T_9	3	R_3	R_3	R_2	R_1	R_1	R_1
T_{10}	3	R_3	R_3	R_3-R_2	R_1	R_1	R_1
T_{11}	3	R_4	R_4-R_3	R_3	R_2	R_2-R_1	R_1
T_{12}	3	R_4	R_4-R_3	R_3	R_3	R_3	R_3
T_{13}	3	R_4	R_4-R_3	R_3	R_3	R_3	R_3
T_{14}	3	R_4	R_4	R_4-R_3	R_3	R_2	R_2
T_{15}	3	R_4	R_3	R_3-R_2	R_2	R_2	R_2

Table 6 : An over rating of selected antagonistic isolates of *Trichodrama* sp. against *Sclerotium rolfsii*

Isolate of *S. rolfsii*	Antagonistic isolates of *Trichoderma* spp.														
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}
S1	—	—	—	—	—	—	—	—	8*	8	—	—	—	—	—
S2	—	—	—	—	—	—	—	—	9	9	—	—	—	—	—
S3	—	—	—	—	—	—	—	—	7	8	—	—	—	—	—
S4	—	—	—	—	—	—	—	—	7	8	7	9	—	—	—
S5	—	—	—	—	—	—	—	—	7	7	9	—	—	—	—

* Days required for attaining R_1 rating of a particular antagonist against specific isolates of *S. rolfsii*

tial in *in-vitro* screaning against *S. rolfsii.* Similarly Bell *et al.* (1982) tested antagonistic activities of *Trichoderma* isolates against different plant pathogens and recorded pathogen-antagonistic interactions. Reports (Elad *et al.,* 1980) showed that while some isolates were highly antagonistic to some pathogen yet there was a clean isolate to isolate variability in the degrees of parasitism.

It could be concluded that there is ample scope to control stem rot or foot rot of betelvine disease through the use of biocontrol agents under field conditions as few antagonistic obtained from the results showed high activity against *S. rolfsii* under *in-vitro* conditions.

The overgrowth by the antagonist under *in-vitro* conditions may be a good criteria of selecting an antagonist provided the isolate showed uniform performance under *in-vitro* conditions.

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